LYSOSOMAL HYDROLASES: CONVERSION OF ACIDIC TO BASIC FORMS BY NEURAMINIDASE

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1. Introduction

A number of hydrolytic enzymes of lysosomal origin occur in multiple forms which differ in such physicochemical properties as solubility, heat and pH stability, pH optima and electrophoretic mobility [1]. Recent studies in this laboratory indicate that essentially all the enzyme proteins of rat kidney and liver lysosomes are glycoproteins, and that at least some of these glycoproteins contain sialic acid [2–4]. The acidic form of lysosomal *N*-acetylhexosaminidase from human spleen [5] and kidney [6] is converted into the basic form by treatment with neuraminidase. We now confirm this finding for rat kidney and show further that the acidic forms of six other hydrolases in rat kidney lysosomes are convertible, in part at least, to more basic forms through the action of neuraminidase.

2. Materials and methods

Renal lysosomes were prepared from tissues of adult albino rat by density gradient centrifugation as described previously [7]. These lysosomal fractions show about a 20-fold increase in β-glucuronidase and acid phosphatase activities over the whole homogenate and are relatively homogeneous by electron microscopy [7]. The lysosomal pellet was suspended in 0.05 M acetate buffer, pH 5, and disrupted by three cycles of freezing and thawing followed by sonication for two min (in 30-sec bursts separated by 30-sec intervals to avoid overheating). The lysosomal lysate was ultracentrifuged at 100,000 *g* for 30 min to give a soluble enzyme fraction. This fraction also contains an acidic lipoprotein [4]. The insoluble residue was suspended in 0.05 M acetate buffer pH 5.0 with 0.2% Triton X-100 and resonicated to release the bound lysosomal enzymes together with additional acidic lipoprotein ([4] and A. Goldstone and H. Koenig, unpublished observations). All operations were carried out at 4°.

Aliquots of the soluble fraction containing about 2 mg of protein were incubated with neuraminidase from *Cl. perfringens* (Type VI enzyme supplied by Sigma), 0.1 mg/ml, or *V. cholerae* (from General Biochemical, 500 units/ml), 0.1 ml/ml, for 3 hr at 37° in 0.05 M citrate–phosphate buffer, pH 5.6, containing 0.02 M $CaCl_2$. For controls tubes containing all ingredients except neuraminidase were similarly incubated or stored at 4° without incubation. Protein was measured by the method of Lowry et al. [8], and free and bound sialic acid by the method of Warren [9]. Aliquots of the soluble fraction so treated and the Triton extract, conatining approximately 0.3 mg of protein per tube, were electrophoresed at 4° on 5% polyacrylamide gels at pH 8.6 for approximately 45 min at 4 mA/tube [10]. Neuraminidase without added lysosomal extracts was electrophoresed as an additional control. None of the hydrolytic activities investigated below was demonstrable in the neuraminidase control. Gels were immediately fixed by immersion in 2% HCHO, pH 5.0, at 4° for 15 min and thence transferred to 0.2 M acetate buffer, pH 5.0, prior to histo-

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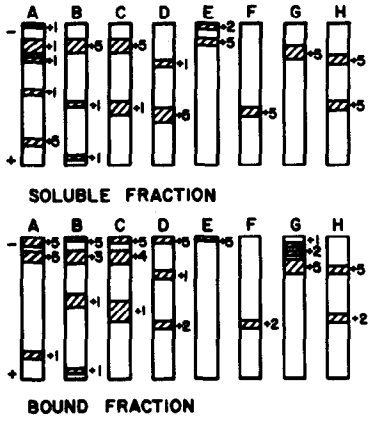

Fig. 1. Gel electrophoretograms of soluble and bound fractions of rat kidney lysosomes stained for acid hydrolase activities. A) Acid phosphatase (α-naphthol phosphate substrate [11]); B) acid DNase [12]; C) acid RNase [12]; D) β-galactosidase (4-methylumbelliferyl-β-D-galactoside substrate [13]); E) *N*-acetylhexosaminidase (4-methylumbelliferyl-β-D-glucosaminide substrate [13]); F) β-glucosidase (4-methylumbelliferyl-β-D-glucoside substrate [13]); G) β-glucuronidase (naphthol AS-BI glucuronide substrate [14]); H) aryl sulfatase (*p*-nitrocatechol sulfate substrate [15]). Relative staining intensities rated on a 1–5 + scale.

chemical staining for enzymes. The following hydrolytic activities were demonstrated by incubation at pH 5 at 37° in appropriate substrates: acid phosphatase [11], acid DNase [12], acid RNase [12], β-galactosidase [13], β-glucuronidase [14], *N*-acetylhexosaminidase [13], β-glucosidase [13], and aryl sulfatase [14]. A Gilford 2410 linear transport was used with a Gilford 2000 absorbance recorder for densitometry of stained gels.

3. Results and discussion

Seven of the eight hydrolases migrated as two or more distinct bands on gel electrophoresis. The acidic form(s) of these enzymes tended to predominate in the soluble fraction, whereas the basic form(s) dominated the bound fraction (fig. 1). The soluble fraction contained 3.1% of protein-bound sialic acid. Incubation of the soluble fraction with neuraminidase cleaved 42–43% of the protein-bound sialic acid and

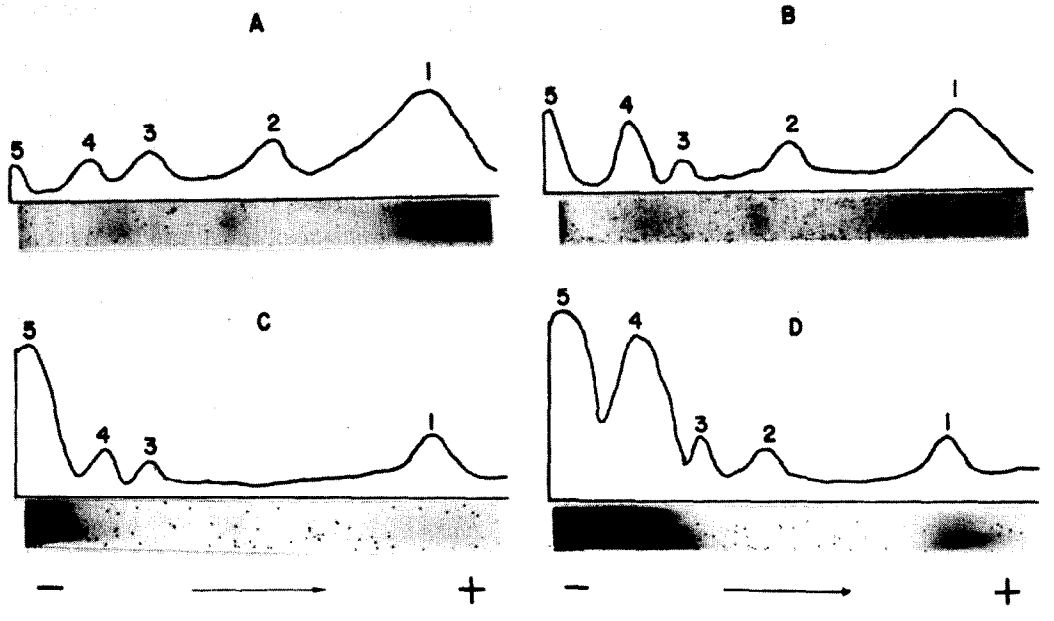

Fig. 2. Gel electrophoretograms and densitometric tracings of soluble and bound lysosomal fractions stained for acid phosphatase activity (α-naphthol phosphate substrate [11]). A) Soluble fraction, unincubated control. B) soluble fraction, incubated control; C) soluble fraction, neuraminidase treatment; D) bound fraction.

reduced the electrophoretic mobility of acidic form(s) of all enzyme activities save one, β-glucosidase. Figs. 2 and 3 show the results for acid phosphatase and β-galactosidase. Incubation of the soluble fraction without added neuraminidase split 15–19% of the protein-bound sialic acid and produced a qualitatively similar, though less marked, shift of enzyme activities toward less anodic forms, presumably due to the activity of endogenous lysosomal neuraminidase.

Acid phosphatase activity in the unincubated soluble fraction appeared as five bands, numbered 1 through 5 in order of decreasing anodic mobility. Band 1 comprised 56%, and band 5, 4.6% of the total activity (fig. 2). The bound enzyme fraction extracted by Triton X-100 consisted mainly of the cathodic components 4 and 5, the more acidic forms representing only about 10% of the bound acid phosphatase activity. Incubation of the soluble fraction with neuraminidase produced an acid phosphatase zymogram pattern which closely resembled that of the bound enzyme in that the cathodic bands 4 and 5 contained over 85% of the total soluble activity. Controls incubated without neuraminidase exhibited a similar, though less marked, shift of acid phosphatase activity from anodic toward cathodic species. Smith and Whitby [16] found that the mobility of the faster electrophoretic components of acid phosphatase from human prostate was reduced by treatment with neuraminidase.

Acid DNase, acid RNase and β-galactosidase (fig. 3) behaved in a similar manner. The soluble fraction contained three acid DNase, two acid RNase and two β-galactosidase components. The bound fraction contained one major cathodic component of each activity, which was absent from the soluble fraction, in addition to small amounts of the more acidic soluble forms (fig. 1). Neuraminidase treatment of the soluble fraction shifted the activity of acid DNase, acid RNase and β-galactoside (fig. 3) from the fast to the slow components, and produced a basic component normally absent from the soluble fraction which had the same mobility as the major cathodic component of the bound fraction. Incubation without enzyme elicited a lesser, but still appreciable, shift of these hydrolytic activities toward less anodic forms. A representative experiment is shown for β-galactosidase in fig. 3.

N-Acetylhexosaminidase activity migrated as two bands, an acidic (A) and a basic (B) form. The soluble fraction contained the A form as the major and the B

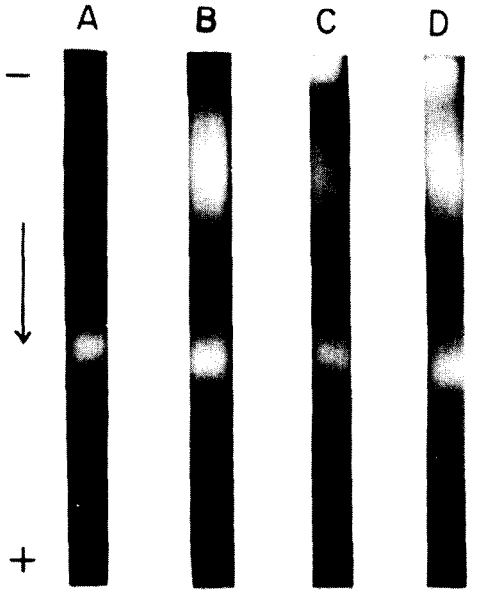

Fig. 3 Gel electrophoretograms of soluble and bound lysosomal fractions stained for β-galactosidase activity (4-methylumbelliferyl-β-D-galactoside substrate [13]) and photographed in near ultraviolet light. A) Soluble fraction, unincubated control; B) soluble fraction, incubated control; C) soluble fraction, neuraminidase treatment; D) bound fraction.

form as the minor component, but only the B form occurred in the bound fraction. In confirmation of earlier reports [5,6] neuraminidase treatment converted the bulk of the A form into the B form. Incubation without added neuraminidase resulted in a smaller conversion of the A into the B form. β-Glucuronidase in the soluble fraction migrated slowly as a single broad band. The bound fraction contained this band together with a cathodic component and a diffuse band of intermediate mobility. Neuraminidase treatment of the soluble fraction elicited the appearance of components of lesser mobility which closely resembled those seen in the bound fraction. Incubation without neuraminidase resulted in the formation of smaller amounts of these more basic forms. β-Glucosidase occurred as a single band of identical mobility in the soluble and bound fractions. Incubation of the soluble fraction with or without neuraminidase had no discernible effect on the electrophoretic migration of this component. Arylsulfatase activity migrated as an acidic (A) and a basic (B) band.

The A and B forms occurred in approximately equal amounts in the soluble fraction, while the B form predominated in the bound fraction. Approximately 40% of the A form was converted into the B form by neuraminidase treatment. Incubation without neuraminidase produced a smaller conversion of the A into the B form. The B form produced by neuraminidase treatment showed increased binding affinity and reacted as arylsulfatase B on biochemical assay (A. Goldstone and H. Koenig, in preparation).

Thus, seven of the eight hydrolases studied in lysosomes from rat kidney occurred as two or more electrophoretic forms. The acidic form(s) of these hydrolases are readily solubilized, together with a more acidic lipoprotein with which they may be complexed in situ in the intact lysosome [7]. The basic form(s) of these enzymes tend to be more firmly bound, apparently to a similar acidic lipoprotein, as both are released into solution by extraction of the lysosomal residue with 0.2% Triton X-100 (A. Goldstone and H. Koenig, unpublished observations). The acidic forms of acid phosphatase, β-galactosidase, and *N*-acetylhexosaminidase underwent an almost total conversion into the basic form(s) under attack by neuraminidase. However, substantial portions of the acidic form(s) of acid DNase, acid RNase, β-glucuronidase and arylsulfatase were resistant to neuraminidase, possibly because the sialic acid residues in these enzyme molecules are not accessible to the action of neuraminidase. In accord with this interpretation, 58% of the protein-bound sialic acid in the soluble lysosomal protein was not split by neuraminidase. It is known that sialic acid linked in terminal position to oligosaccharide chains is readily cleaved by neuraminidase, whereas internally located sialic acid residues resist enzymatic cleavage [17].

These observations confirm the glycoprotein nature of the lysosomal hydrolases reported earlier [2–4], and suggest further that the acidic form(s) of these hydrolases are sialoglycoproteins. The biological significance on the multiple forms of lysosomal enzymes is obscure. It seems possible that these forms may differ in their hydrolytic activities under physiological conditions. The acidic form(s) of the lysosomal hydrolases are more readily released into solution from the particulate state than the basic forms and thus may be more effective in the intracellular digestion of insoluble macromolecules. In this regard it is pertinent to note the selective absence of the acidic form of a lysosomal hydrolase in two lipid storage diseases. In Tay-Sachs disease, a fatal hereditary cerebral degeneration involving the accumulation of a specific ganglioside, GM_2, hexosaminidase A is absent in tissues and body fluids while hexosaminidase B is present in normal or increased amounts [18,19]. In metachromatic leukodystrophy, a genetic degenerative disease of the nervous system in which another glycosphingolipid, cerebroside sulfate, accumulates, there is a generalized deficiency of arylsulfatase A whereas arylsulfatase B is present in normal or elevated amounts [20,21]. If, as seems possible from our results and those of Robinson et al. [5,6], hexosaminidase A and arylsulfatase A differ from the corresponding B forms in the presence of sialic acid residues, patients with Tay-Sachs disease and metachromatic leukodystrophy may lack the specific sialyl transferases to produce these A forms. An increased level of endogenous neuraminidase activity would not readily account for the genetic absence of the acidic form of a single hydrolase.

Acknowledgments

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